Inflammatory exudate produced 24 h after i.p. injection in normal mice by supernatants from sensitized human lymphocyte cultures and by control supernatants

Culture supernatants		Stimulated cells	Unstimulated cells	Killed cells	Medium and antigen	Medium control
Cell Count $\times 10^3$/ml		7.12 ± 2.3	2.3 ± 0.9	2.3 ± 0.6	1.9 ± 0.9	3.4 ± 2
t-test			$p < 0.01$	$p < 0.01$	$p < 0.01$	< 0.05
Differential	Polymorphs	4	0	0	0	0
Count	Lymphocytes	74	88	91	85	95
Percent	Monocytes	22	12	9	15	5

Unconcentrated supernatants from the stimulated and control leukocyte cultures were injected i.p. in 1 ml aliquots into normal C57 BL mice anaesthetized with ether. There were 6 animals in each treatment group. The mice were sacrificed at 8, 24 and 48 h after the initial injection, each animal being injected i.p. with 1 ml of warm RPMI 1640 1 h prior to sacrifice. Subsequently, reactions were assessed at 24 h only. After sacrifice, any fluid contained within the peritoneal cavity was withdrawn using a blood diluting pipette, a drop of the fluid was smeared upon a slide for later fixation and staining and the remaining fluid was used to carry out a white cell count using a hemocytometer.

Results and discussion. The i.p. injection in mice of supernatants from the human lymphocyte cultures, as indicated in the figure, resulted in a peak in the inflammatory exudate produced that occurred at 24 h with supernatants from sensitized lymphocytes cultured with PPD. At other times and with control supernatants, the cellularity of the exudate remained approximately the same.

In further experiments, the nature of the exudate occurring 24 h after i.p. injection of the culture supernatants was examined. It was found that supernatants from sensitized human lymphocyte cultures stimulated with PPD produced exudates with significantly greater cellularity than did those from control supernatants (table). The differential counts obtained from fixed and stained smears of the exudates did not appear to differ markedly between groups. There were more monocytes and fewer lymphocytes, and some polymorphonuclear leukocytes present in the exudates developing in response to supernatants from stimulated cultures, as compared with the exudates induced by control supernatants. Supernatants from a number of donors with approximately equal tuberculin sensitivity were assessed using the assay system. All supernatants induced an inflammatory exudate of similar cellularity, in that cell counts of exudates induced by stimulated lymphocyte culture supernatants were approximately 3 times those induced by unstimulated control supernatants. A stimulated culture supernatant concentrated seven times by lyophilization produced an exudate with 7 times the cellularity of that induced by the unconcentrated supernatant (42×10^3 cells/ml compared to 5.92×10^3 cells/ml).

There thus appeared to be evidence for the presence of inflammatory factors in PPD stimulated lymphocyte culture supernatants. These supernatants produced an exudate peaking at 24 h. In addition, there was a tendency for such supernatants to induce an inflammatory response containing more monocytes and polymorphs than that seen with contol supernatants. This would not be unexpected if the active supernatants contain factors such as the macrophage migration inhibition factor (MIF)[7] and the polymophonuclear leukocyte inhibitory factor (LIF)[8] that have been demonstrated by in vitro techniques. It should be stressed that the ability of these supernatants to stimulate an inflammatory exudate may or may not reflect SRF activity. Nevertheless, the data presented do demonstrate that soluble factors from human lymphocytes will induce an inflammatory response in the mouse that may be characterized and quantitated by this assay system.

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Acceleration of methemoglobin reduction in erythrocytes by selenium

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Summary. Selenium accelerated the reduction of methemoglobin in erythrocytes. Its mode of action is suggested as a catalysis of the methemoglobin reduction by glutathione.

It was reported that dietary selenium prevents not only hemolysis, but also oxidation of hemoglobin to methemoglobin induced by hydrogen peroxide or ascorbic acid[3–5]. Since it was recently demonstrated that selenium forms an integral part of the glutathione peroxidase molecule[5,6] whose activity is proportional to the amount of dietary selenium[7], the prevention of oxidative damage of erythrocytes by selenium has been suggested to be associated with this enzyme activity in the cells. This paper reports the role of selenium in preventing oxidative damage of hemoglobin, showing that selenium accelerates the reduction of methemoglobin in intact rat erythrocytes.

Materials and methods. Male Sprague-Dawley rats were decapitated and their blood was collected in heparinized tubes and centrifuged at $500 \times g$ for 10 min at 4 °C. The plasma and buffy coat were removed and the erythrocytes were washed 3 times with isotonic phosphate buf-

fered saline (pH 7.4). To convert the hemoglobin to methemoglobin, a suspension of erythrocytes was incubated in the presence of $NaNO_2$ and glucose at 37 °C for 30 min (Final mixture: 5% erythrocytes, 24 mM $NaNO_2$, and 10 mM glucose in 0.107 M phosphate buffer (pH 7.4). Then the erythrocytes were washed 6 times with isotonic phosphate buffered saline (pH 7.4). In this way, about 90% of the hemoglobin was converted to methemoglobin, as determined by the method of Evelyn and Malloy[8]. The basal mixture used to study the reduction of methemoglobin consisted of 25% erythrocytes plus 10 mM glucose in isotonic phosphate buffered saline (pH 7.4). Hemoglobin was measured by the cyanmethemoglobin technique. Glutathione was determined as described by Ellman[9].

Results. The table shows the effects of various metals on the reduction of methemoglobin in rat erythrocytes. The reduction of methemoglobin was greatly stimulated by 10^{-5} M selenite and slightly by selenate and tellurite. However, it was not affected by organic seleno-compounds, such as selenomethionine and selenocystine, or by other metal ions.

This reduction of methemoglobin by selenium could be observed by eye, because oxyhemoglobin is bright red, whereas methemoglobin is dark brown. The absorption spectrum of erythrocytes incubated with selenite changed to that of oxyhemoglobin, showing increase in absorbance at 542 and 578 nm and decrease at 630 nm (figure 1). Selenite 10^{-5} to 10^{-6} M caused progressive increase in the methemoglobin reduction, and significant decrease in the glutathione level within 1 h (figure 2).

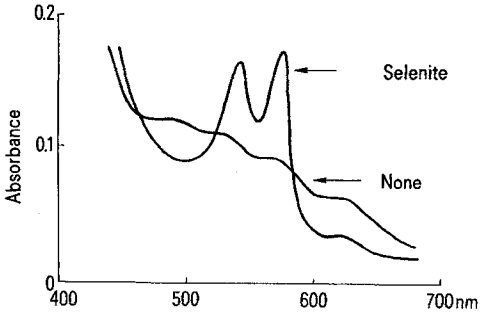

Fig. 1. Absorption spectra of nitrite-treated erythrocytes with or without sodium selenite (10^{-5} M) after incubation for 3 h at 37 °C.

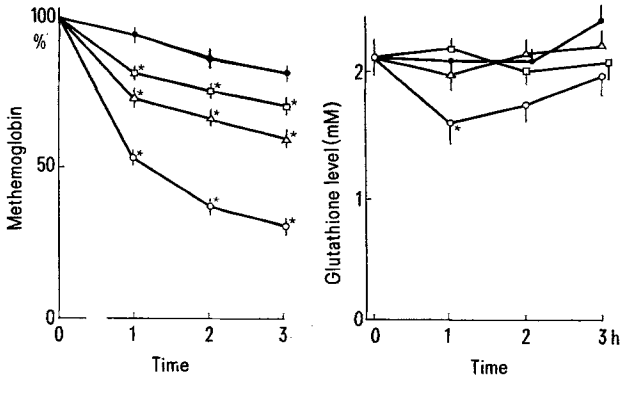

Fig. 2. Time course of changes in methemoglobin and glutathione by sodium selenite in nitrite-treated erythrocytes from rats. —●— No addition, —○— Sodium selenite 10^{-5} M, —△— 3×10^{-6} M, —□— 10^{-6} M. Points and bars are means ± SE of values in 5 separate experiments. * $p < 0.01$.

Selenite is reported to catalyze oxidation of glutathione[10]. So, we studied the influence of glutathione on the reduction of methemoglobin with selenite. When a hemolysate of nitrite-treated erythrocytes was passed through Sephadex G-25 column, the acceleration of methemoglobin reduction by selenite was abolished completely, and it was restored by addition of 1 mM glutathione. However, when erythrocytes had been subjected to hydrogen peroxide diffusion, which decreases the glutathione level by half, acceleration of methemoglobin reduction by selenite was still observed. These findings suggest that selenium may act as a catalyst of methemoglobin reduction by glutathione.

Discussion. The biological role of the selenium moiety of selenoproteins is unknown. It may participate in electron transfer, because it is an essential constituent of several enzymes catalyzing oxidation or reduction[5,6,11,12]. Moreover, selenium was suggested to be important in other electron transport systems, such as those of liver micro-

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Effects of various metals on the reduction of methemoglobin in nitrite-treated erythrocytes from rats

Metal	Methemoglobin (g/100 ml of erythrocytes)
None	20.66 ± 0.52
Na_2SeO_3	11.30 ± 0.68*
Na_2SeO_4	16.60 ± 0.54*
Selenomethionine	20.96 ± 0.60
Selenocystine	20.42 ± 0.56
Na_2TeO_3	17.36 ± 0.70*
Na_2SO_3	21.45 ± 0.70
$CuCl_2$	21.09 ± 0.70
$FeCl_3$	21.04 ± 0.75
$CoCl_2$	20.84 ± 0.71
$MnSO_4$	20.72 ± 0.63

The initial methemoglobin level was 23.30 ± 0.94 g/100 ml of erythrocytes. The reaction was initiated by adding the various metals indicated and was carried out for 1 h at 37 °C. The final concentration of metals was 10^{-5} M. Values are the means ± SE of those in 5 separate experiments.
* $p < 0.01$.

somes[13,14] and mitochondria[15,16]. Recently, selenite has been reported to react with glutathione forming seleno-intermediates, such as seleno-persulfide and -trisulfide[17]. Thus a seleno-intermediate may facilitate the transfer of electrons from glutathione to methemoglobin, causing reduction of the latter.

We found that the mean selenium level in rat plasma was 3.45×10^{-6} M. Thus the acceleration of methemoglobin reduction by selenium may be a physiological phenomenon. The oxidative damage of hemoglobin observed in selenium deficient animals may also be due to lack of selenium-catalyzed reduction of methemoglobin as well as deficiency of glutathione peroxidase.

From in vitro studies in the absence of selenium, Scott et al.[18] estimated that methemoglobin reduction by glutathione represents 13% of the total reduction. However, our data suggests that under physiological conditions, the capacity of glutathione to reduce methemoglobin in the presence of selenium may be greater than this.

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Aggregation pheromones in 2 Australian hard ticks, Ixodes holocyclus and Aponomma concolor

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Summary. The presence of an aggregation pheromone has been demonstrated for the first time in indigenous Australian ticks. Filter paper discs exposed to either Australian paralysis ticks Ixodes holocyclus or echidna ticks Aponomma concolor showed inter-sex or intra-sex attraction for ticks of their own species. Nymphal exuviae of Ap. concolor were highly attractive to adult ticks. Discs were significantly attractive to I. holocyclus at distances up to 80 cm.

An aggregation pheromone has been demonstrated in argasid ticks[3–5], in an ixodid tick[6] and sex pheromones in a number of ixodid tick species[7–10]. Amblyomma americanum and A. maculatum[5,7], Dermacentor variabilis and D. andersoni[7,10], males are attracted by pheromones secreted by females while A. maculatum females[8,9] and A. hebraeum nymphs[7] are attracted to secretions of attached males. The principal hosts of the paralysis tick, Ixodes holocyclus, are 3 species of bandicoot[11] (omnivorous marsupials – Perameles nasuta, Isoodon obesulus and I. macrourus) but it attaches to a large number of hosts including cattle and domestic pets, causing paralysis and some fatalities. The echidna tick, Aponomma concolor, appears to be found only on the 2 echidna[11] species (monotremes – Tachyglossus aculeatus and T. setosus) and its effect on the host is unknown. Both ticks are almost certainly confined to Australia.

Materials and methods. 1. General aggregation. Tests were carried out using unfed ticks by the 'petri-dish' method essentially as described by Leahy et al.[3], except that in some experiments, 4 sectors were used instead of 8. I. holocyclus ticks were humidified during tests by attaching a moistened filter paper inside the lid and illuminating artificially but Ap. concolor ticks were neither humidified nor illuminated[12]. Filter paper discs were held with adult I. holocyclus[13] for either 7 days after ecdysis, or between days 7–21 or 42–63 after ecdysis, and then assayed as centres of aggregation with ticks aged 7, 21 or 63 days respectively. Discs were also held with adult Ap. concolor[13] between days 36–42, days 70–126, days 166–168 or days 231–238 after ecdysis and assayed with ticks aged 42, 126, 168 or 238 days respectively. In addition, assays of saline extracted discs[14] and of nymphal exuviae were made for Ap. concolor. Significance

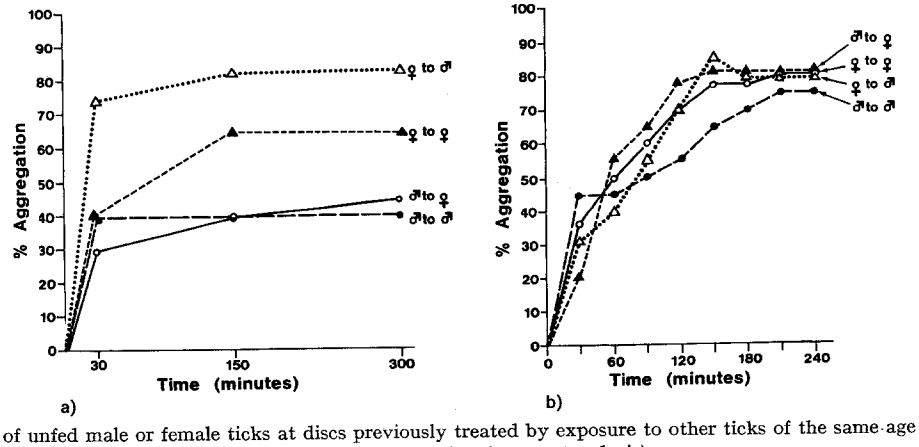

Fig. 1. Aggregation of unfed male or female ticks at discs previously treated by exposure to other ticks of the same age (approximately 20 ticks per test). *a* I. holocyclus (7 days post ecdysis). *b* Ap. concolor (42 days post ecdysis).